Plant Physiol. (1988) 88, 540–542
0032-0889/88/88/0540/03/$01.00/0

Communication

Phytochrome Mediated Regulation of Sucrose Phosphate Synthase Activity in Maize

Received for publication April 4, 1988 and in revised form May 31, 1988

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ABSTRACT

The extractable activity of sucrose phosphate synthase was determined in etiolated seedlings of maize (*Zea mays* L.), soybean (*Glycine max* [L.] Merr.), and sugar beet (*Beta vulgaris* L.) following treatments of changing light quality. A 30-minute illumination of 30 microeinsteins per square meter per second white light produced a three-fold increase in sucrose phosphate synthase activity at 2 hours postillumination when compared to seedlings maintained in total darkness. Etiolated maize seedlings treated with 3.6 microeinsteins per square meter per second of red and far-red light showed a 50% increase and a 50% decrease in sucrose phosphate synthase activity, respectively, when compared to etiolated maize seedlings treated with white light. Maize seedlings exposed for 30 minutes to red followed by 30 minutes to far-red showed an initial increase in sucrose phosphate synthase activity followed by a rapid decrease to control level. Neither soybean or sugar beet sucrose phosphate synthase responded to the 30-minute illumination of white light. Phytochrome is involved in sucrose phosphate synthase regulation in maize, whereas it is not responsible for changes in sucrose phosphate synthase activity in soybean or sugar beet.

Sucrose-P synthase appears to be a major control point in sucrose formation during photoassimilation of CO_2 (10). Sucrose synthesis is apparently the only physiological function of sucrose-P synthase, with the equilibrium constant for sucrose synthesis about 2 (1). This makes sucrose-P synthase a focal point for studying regulation of sucrose metabolism and partitioning.

Vidal and Gadal (18) and Vidal *et al.* (19) found that two isoforms of PEPC[1] were present in etiolated leaves of sorghum. One form, found in the eitolated leaves, was designated E-PEPC. The other form found in greening leaves was designated G-PERPC and occurred only after etiolated leaves were exposed to light. Later, Thomas *et al.* (16) showed that the activity of G-PEPC was sensitive to red and far-red treatments. A 10-min exposure to red stimulated G-PEPC, whereas far-red (either alone or following a red treatment) caused a substantial inhibition of the G-PEPC isoform. They concluded from these experiments that phytochrome was the photoreceptor triggering changes in enzyme activity.

The present study was designed to examine the role of light quality on the extractable activity of sucrose-P synthase in maize, soybean, and sugar beets. This was done using 10-day-old seedlings of each species grown in total darkness and illuminating them with white light, red, far-red, or red followed by far red.

MATERIALS AND METHODS

Plant Materials. Maize (*Zea mays* L., cv FS854), soybean (*Glycine max.* [L.] Merr., cv Pioneer 1082), and sugar beet (*Beta vulgaris* L., cv Great Western multigerm hybrid) seeds were germinated in darkness then planted in 7 cm × 7 cm plastic pots containing vermiculite. These pots were placed into an incubation chamber of total darkness. Chambers were maintained at 20°C with 80% RH. Once seedlings had emerged through the soil surface they were considered 1 d old. Ten-d-old seedlings were used for light treatments and enzyme assay.

Treatments. For light exposure, 10-d-old seedlings were placed in a 43.5 × 29.5 × 30 cm (LWH) styrofoam box covered in Al foil. Light treatments were administered through a 4.3 × 4.5 cm opening at the top of the box. To obtain the desired wavelength, filters in the red (673 nm, half-bandwidth of 36 nm) and far-red (760 nm, half-bandwidth of 21 nm) range were placed in the 4.3 × 4.5 cm^2 opening. White light, provided by an incandescent bulb (PPFD of 30 $\mu E\ m^{-2}\ s^{-1}$), was passed through a ferrous ammonium sulfate filter (8) and then through the red or far-red filters to illuminate the seedlings within the box. The PPFD of the red treatment was only 3.6 $\mu E\ m^{-2}\ s^{-1}$, once it passed through the red filter. Each illumination period was 30 min in length. At the end of each illumination period plants were returned to the darkened growth chamber for 1, 2, or 4 h. At the end of the postillumination dark periods, plants were harvested for enzyme assay. Treatments included dark control, white light, red, far-red, or red followed by far-red for red-far-red reversal.

Enzyme Assay. Fresh leaf material was ground in a cold mortar and pestle in an extraction buffer (8 mL buffer/g fresh weight) containing 25 mM Hepes/KOH (pH 7.5), 5 mM $MgSO_4$, 15 mM KCl, 2 mM sodium diethyldithiocarbamate, 2 mg/mL PVP (soluble), and 5 mM freshly added β-mercaptoethanol. The extract was filtered through eight layers of cheesecloth and the filtrate was centrifuged at 12,000 rpm for 10 min. A 1-mL aliquot of supernatant was passed through a prespun 5-mL G-50 Sephadex column packed into a 5-mL syringe. This column was centrifuged at 475*g* for 30 s, and the eluant was used for enzyme assay.

Sucrose-P synthase was assayed by measurement of sucrose produced from fructose 6-P plus UDPG. The reaction mixture contained 250 μL leaf extract and 50 μL 100 mM fructose 6-P, 100 mM UDPG, 40 mM Hepes/KOH (pH 7.5), and 10 mM $MgSO_4$.

Mixtures were incubated at 25°C and the reactions were ter-

[1] Abbreviations: PEPC, phosphoenolpyruvate carboxylase; G6P, glucose 6-phosphate; UDPG, uridine 5′-diphosphoglucose; PPFD, photosynthetic photon flux density.

minated by mixing a 50-μL aliquot of enzyme preparation with 50μL of 30% KOH. Points were taken at 5 min and 10 min. Hexoses were destroyed by placing the tubes in boiling water for 10 min. The tubes were cooled, 1 mL of anthrone reagent (76 mL H_2SO_4, 30 mL H_2O, and 150 mg anthrone) was added, the mixture was incubated at 37°C for 20 min, and the A_{620} was measured (17). Protein was determined by a modified method as described by Peterson (7).

RESULTS

Maize Sucrose Phosphate Synthase. Etiolated maize seedlings were illuminated with 30 μE m^{-2} s^{-1} white light for 30 min and then returned to the darkened chamber. Leaf samples were then taken at 1, 2, and 4 h postillumination. When compared to those plants which were maintained in total darkness, extractable sucrose-P synthase activity increased threefold by 1 h (Fig. 1A). The activity remained constant for an additional hour and then decreased to about 50% of maximal 4 h postillumination.

The rate of sucrose synthesis obtained in these etiolated maize seedlings at 30 μE m^{-1} s^{-1} of approximately 30 nmol sucrose min^{-1} mg^{-1} protein was about 60% lower than those rates obtained by Sicher and Kremer (12) with 17-d-old light-grown maize plants. This most likely resulted from being grown initially and entirely in the dark.

The involvement of phytochrome was tested by treating 10-d-old dark-grown etiolated maize seedlings with a 30-min illumination of red or 30-min illumination for far-red. Red light increased enzyme activity about 56% over the dark control (Fig. 1B). The increase in sucrose-P synthase activity resulting from the red light treatment was only 50% of that seen with white light. This was due to low PPFD (3.6 *versus* 30 μE m^{-2} s^{-1}) available through the red filter. This was only 1/10 that available without the filter. A 30-min illumination of far-red light resulted in a 43% decrease in sucrose-P synthase activity 1 h postillumination and a 61% decrease in sucrose-P synthase activity 4 h postillumination (Fig. 1B). These are the expected changes in sucrose-P synthase activity if phytochrome is involved.

Figure 1C shows the effect of a 30-min illumination of far-red following an initial 30-min illumination of red. As in Figure 1B, sucrose-P synthase increases with a red treatment. However, those seedlings which received an additional far-red treatment showed a reversal in sucrose-P synthase activity by 2 h postillumination, resulting in an approximately 50% decrease in enzyme activity. These data demonstrate the reversibility of sucrose-P synthase activity by illumination with red followed by far-red and further supports the hypothesis that phytochrome may be involved in light dependent changes in sucrose-P synthase activity in maize (16).

Soybean and Sugar Beet Sucrose Phosphate Synthase. Soybean seedlings, illuminated with 30 min of white light, showed no increase in extractable sucrose-P synthase activity over seedlings maintained in total darkness (Table I). Table I shows the same results for sugar beet seedlings illuminated for 30 min with white light. There appears to be no difference in sucrose-P synthase activity between illuminated soybean or sugar beet plant and those maintained in darkness for the same period. These rates for soybean, however, were 80% lower than those observed in mature soybeans (12) and 50% lower than in mature sugar beet observed by myself.

DISCUSSION

A sensitivity to red light and reversal by far red light in maize indicate that the activity of sucrose-P synthase, a critical enzyme in sucrose metabolism, is modulated differently in maize than in soybean and sugar beet. This may reflect a fundamental difference between monocotyledonous plants versus dicotyledonous plants.

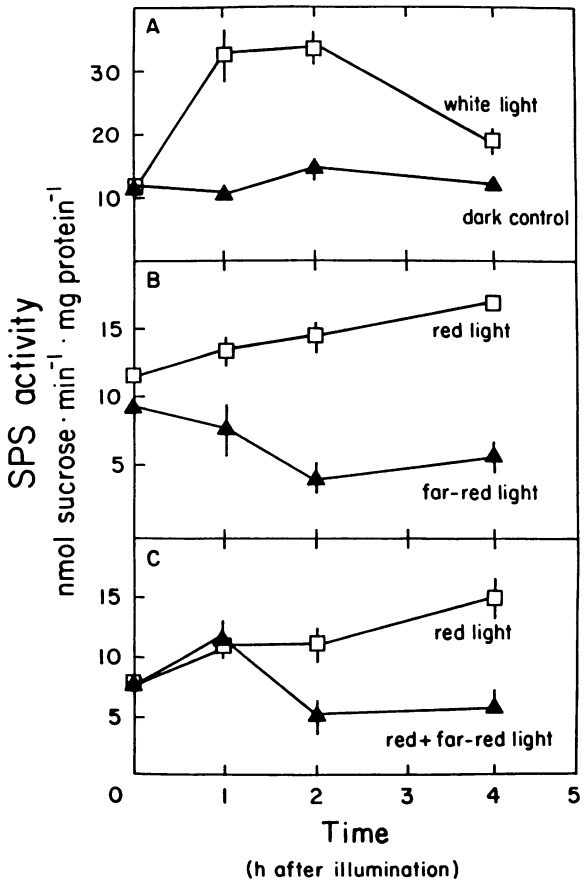

FIG. 1. A, Sucrose-P synthase (SPS) activity in 10-d-old etiolated corn seedlings illuminated with white light or maintained in continuous darkness. Plants exposed to white light were returned to darkness and enzyme activity was then determined 1, 2, and 4 h postillumination: (□), 30 min white light; (▲), continuous darkness. Data represent means of three experiments. B, Sucrose-P synthase activity in 10-d-old etiolated corn seedlings illuminated with red or far-red. Plants were returned to darkness at the end of light treatment. Enzyme activity was determined 1, 2, and 4 h postillumination: (□) 30 min red; (▲) 30 min far-red. Data represent means of three experiments. C, Sucrose-P synthase activity in 10-d-old etiolated corn seedlings illuminated with red followed by illumination with far-red. All plants were illuminated with 30 min red (□). A subset of red treated plants was removed and the remainder were illuminated with 30 min far-red (▲). Plants were returned to the dark after each light treatment. Enzyme activity was determined 1, 2, and 4 h postillumination. Data represent the means of three experiments.

Maize and barley sucrose-P synthase activity is modulated by light as is also the case for *Lolium* (9, 12, 13). This regulation, or in the case of these experiments the induction of sucrose-P synthase, appears to be mediated by the action of phytochrome, either through modification of existing proteins or through *de novo* synthesis of new proteins. Based on studies with partially purified maize leaf sucrose-P synthase, Kremer and Sicher (12) argue that *de novo* synthesis of new proteins is probably unlikely. They felt that regulation of sucrose-P synthase in these species is more likely through altered affinities for the substrates UDPG and fructose 6-P. If phytochrome is involved, then the enzyme itself and not the photosynthetic apparatus may play a more important role in carbon partitioning in monocotyledonous

Table I. *Sucrose-P Synthase Activity in Etiolated Soybean and Sugar Beet Seedlings*

Ten-d-old seedlings were illuminated with white light or maintained in continuous darkness. Plants illuminated with white light (30 μE m^{-1} s^{-1} of PPFD) were returned to darkness and enzyme activity was determined 2 and 4 h postillumination. Data represent means of three experiments.

Species	Sucrose-P Synthase Activity		
	Time post-illumination	Dark control	White light
	h	*nmol sucrose min^{-1} mg^{-1} protein*	
Soybean	0	10.8 ± 3.6	
	2	12.0 ± 3.5	10.8 ± 0.3
	4	13.9 ± 4.0	9.8 ± 0.6
Sugar beet	0	11.1 ± 1.5	
	2	16.0 ± 1.3	14.1 ± 3.2
	4	13.9 ± 1.1	13.1 ± 4.0

plants by modulating the relationship between triose-P and Pi in the cytosol (feedback) (3, 4).

Sucrose-P synthase activity in soybeans and sugar beets, on the other hand, does not seem to be as closely related to light/dark transitions and therefore is probably not modulated by light or phytochrome. It appears that sucrose-P synthase in these two species is modulated more by diurnal changes not associated with the light/dark transitions (10, 11). Sucrose-P synthase activity may be regulated in dicotyledonous plants species by photosynthetically altered concentrations of certain metabolites in the cytosol and therefore be more closely associated with the photosynthetic apparatus. Doehlert and Huber (2) showed that sucrose-P synthase activity is regulated by changes in cytosolic G6P and Pi along with triose-P availability (14). As photosynthesis begins, the initial activity of sucrose-P synthase in soybean (6, 11) and sugar beet (TL Vassey, personal data) is high. Later in the photoperiod the activity falls to a lower activity and continues into the dark period. A second peak in sucrose-P synthase activity is observed during the dark period and this is unrelated to photosynthesis. This dark period increase in sucrose-P synthase activity could be associated with increased starch degradation and increases in metabolites of sucrose formation released into the cytosol from the chloroplast.

Although the mechanism of sucrose-P synthase regulation is not yet understood it is apparent that this enzyme is correlated with photosynthetic rate or the availability of various cytosolic metabolites such as triose-P, G6P and the inhibitor fructose-2,6-bisP (5, 15). In the monocotyledonous plants studied there is a strong relationship between sucrose-P synthase activity and changes in light quantity and quality associated with phytochrome regulation. In the dicotyledonous plants studied, however, this relationship does not exist and the activity of sucrose-P synthase appears to be modulated by a circadian rhythm.

Acknowledgments—The author is grateful to David H. Brandwein for technical assistance in this project and to Drs. Mary A. Schuler and Thomas W. Jacobs for critical use of laboratory space.

LITERATURE CITED

1. Barber GA 1985 The equilibrium of the reaction catalyzed by sucrose phosphate synthase. Plant Physiol 79: 1127–1128
2. Doehlert DC, SC Huber 1983 Regulation of spinach leaf sucrose phosphate synthase by glucose-6-phosphate, inorganic phosphate and pH. Plant Physiol 73: 939–994
3. Herold A 1980 Regulation of photosynthesis by sink activity—the missing link. New Phytol 86: 131–144
4. Huber SC, DW Israel 1982 Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean (*Glycine max.* Merr.) leaves. Plant Physiol 69: 691–696
5. Kerr PS, SC Huber 1987 Coordinate control of sucrose formation in soybean leaves by sucrose phosphate synthase and fructose-2,6-bisphosphate. Planta 170: 197–204
6. Kerr PS, TW Rufty, SC Huber 1985 Endogenous rhythms in photosynthesis, sucrose phosphate synthase activity, and stomatal resistance in leaves of soybean (*Glycine max* [L.] Merr.). Plant Physiol 77: 275–280
7. Peterson GL 1971 A simplification of the protein assay method of Lowery et al which is more generally applicable. Anal Biochem 83: 346–356
8. Pfund AH 1939 Transparent and opaque screens for the near infra-red. J Opt Soc Am 29: 56–58
9. Pollock CJ, TH Housley 1985 Light-induced increase in sucrose phosphate synthase activity in leaves of *Lolium temulentum.* Ann Bot 55: 593–596
10. Rufty TW, SC Huber 1983 Changes in starch formation and activities of sucrose phosphate synthase and cytoplasmic fructose-1,6-bisphosphatase in response to source-sink alterations. Plant Physiol 72: 474–480
11. Rufty TW, PS Kerr, SC Huber 1983 Characterization of diurnal changes in activities of enzymes involved in sucrose biosynthesis. Plant Physiol 733: 428–433
12. Sicher RC, DF Kremer 1985 Possible control of maize leaf sucrose-phosphate synthase activity by light modulation. Plant Physiol 79: 695–698
13. Sicher RC, DF Kremer 1984 Changes of sucrose phosphate synthase activity in barley primary leaves during light/dark transitions. Plant Physiol 76: 910–912
14. Stitt M, B Herzog, HW Heldt 1984 Control of photosynthetic sucrose synthesis by fructose 2,6-bisphosphate. I. Coordination of CO2 fixation and sucrose synthesis. Plant Physiol 75: 548–553
15. Stitt M 1985 Fine control of sucrose synthesis by fructose-2,6-bisphosphate. *In* RL Heath, J Preiss, eds, Regulation of Carbon Partitioning in Photosynthetic Tissue. American Society of Plant Physiologists, Rockville, MD, pp 109–126
16. Thomas M, C Crétin, E Keryer, J Vidal, P Gadal 1987 Photocontrol of sorghum leaf phosphoenolpyruvate carboxylase. Characterization of messenger RNA and of photoreceptor. Plant Physiol 85: 243–246
17. Van Handel E 1983 Direct microdetermination of sucrose. Anal Biochem 22: 280–283
18. Vidal J, P Gadal 1983 Influence of light on phosphoenol pyruvate carboxylase in sorghum leaves. I. Identification and properties of two isoforms. Physiol Plant 57: 119–123
19. Vidal J, G Godbillon, P Gadal 1983 Influence of light on phosphoenol pyruvate carboxylase in sorghum leaves. II. Immunochemical study. Physiol Plant 57: 124–128